(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) Its absorptivity at the absorption maximum of 345 nanometers relative to that of the methacycline working standard similarly treated is 92.4±4 percent.

(vi) It gives a positive result to the identity test for methacycline hydrochloride.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams of methacycline per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 100 milliliters of 0.01*N* methanolic hydrochloric acid. Transfer a 10-milliliter aliquot to a 250-milliliter volumetric flask and dilute to volume with 0.01*N* methanolic hydrochloric acid. Using a suitable spectrphotometer and 0.01*N* methanolic hydrochloric acid as the blank, scan the absorption spectrum between the wavelengths of 250 and 400 nanometers. Determine the absorbance of each solution at the maxima, ca. 345 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

Percent relative absorptivity=(Absorbance of sample×weight in milligrams of standards×potency of standard in micrograms per milligram)/(Absorbance of standard×weight in milligrams of sample×10)

(6) *Identity.* The absorption spectrum between the wavelength of 250 and 400 nanometers, determined as directed in paragraph (b)(5) of this section, compares qualitatively with that of the methacycline standard.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§446.60 Minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride is [4S-(4α,4aα,5aα,12aα)]-4,7-bis (dimethylamino)-1,4,4a,5,5a,6,11, - 12a-octahydro-3,10,12, - 12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 890 micrograms per milligram and not more than 950 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams of minocycline per milliliter is not less than 3.5 and not more than 4.5.

(v) Its epi-minocycline content is not more than 1.2 percent.

(vi) It gives a positive identity test for minocycline hydrochloride.

(vii) It is crystalline.

(viii) Its residue on ignition is not more than 0.15 percent.

(ix) The absorptivity at 560 nanometers of an aqueous solution containing 10 milligrams of minocycline hydrochloride per milliliter is not more than 0.006.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, epiminocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Minocycline potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 4.6-millimeter × 3-centimeter guard column containing 10-micrometer diameter RP–8 Lichrosorb, a 4.6-millimeter × 15-centimeter analytical column packed with octyl silane chemically bonded to porous microsilica particles, 5 micrometers in diameter, a flow rate of 2.9 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.1 M Disodium ethylenediamine-tetraacetate (EDTA).* Accurately weigh 37.22 grams of disodium ethylenediaminetetraacetate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*b*) *0.2 M Ammonium oxalate.* Accurately weigh 28.42 grams of ammonium oxalate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*c*) *Mobile phase.* Mix 250 milliliters of dimethylformamide, 200 milliliters of 0.1*M* disodium ethylenediaminetetraacetate and 550 milliliters of 0.2*M* ammonium oxalate. (5:4:11). Allow the solution to cool to room temperature and then adjust the pH to 6.2 to 6.3 with 0.4*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chomatographic pumping system.

(ii) *Preparations of working standard, sample and resolution testing solutions*—(*a*) *Working standard solution.* Dissolve an accurately weighed portion of the minocycline hydrocholoride working standard with sufficient mobile phase (prepared as described in paragraph (b)(1)(i)(*c*) of this section) to obtain a solution containing 500 micrograms of minocycline activity per milliliter. Use this standard solution within 3 hours of preparation.

(*b*) *Sample solution.* Dissolve an accurately weighed sample in sufficient mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparations.

(iii) *System suitability requirements*—(*a*) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s)is satisfactory if it is not less than 0.9 and not more than 1.35.

(*b*) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in § 436.216(c)(2) calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;

n=number of theoretical plates; and

d_p=Average diameter of the particles in analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 50 for the minocycline peak.

(*c*) *Resolution.* Dissolve 50 milligrams of minocycline hydrochloride in 25 milliliters of deionized water. Pipet 5 milliliters of this solution into a 25-milliliter volumetric flask and heat on a steam bath for 60 minutes. Transfer the contents of the flask to a small beaker and evaporate to dryness. Dissolve the residue in mobile phase, transfer to a 25-milliliter volumetric flask, dilute to mark with mobile phase, mix, and filter through Whatman No. 1 filter

paper. Use this solution to determine the resolution factor. The resolution (R) between the peaks for minocycline and epi-minocycline is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(*e*) *Capacity factor (k′).* Calculate the capacity factor (*k′*) for minocycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of minocycline in minutes; and

t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (*k′*) for minocycline is satisfactory if it is not less than 6.2 and not more than 11.5.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—Calculate the micrograms of minocycline per milligram of sample as follows:

$$\text{Micrograms of minocycline per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;

P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

C_u=Milligrams of minocycline sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

(5) *Epi-minocycline content.* Proceed as directed in paragraph (b)(1) of this section. Calculate the epi-minocycline content as follows:

$$\text{Percent Epi-minocycline} = \frac{(A_{epi}) \times 100}{(A_{total})}$$

where:

A_{epi}=Area of the epi-minocycline peak in the chromatogram of the sample; and

A_{total}=The sum of the areas of all the peaks eluting after the solvent front.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter.

(9) *Absorptivity.* Accurately weigh about 1 gram of sample into a 100-milliliter volumetric flask, dissolve, and dilute to mark with deionized water. Determine the absorbance of this solution on a suitable spectrophotometer at 560 nanometers (nm) using 5-centimeter cells with water in the reference cell. Calculate the absorptivity as follows:

$$\text{Absorptivity at 560 nm} = \frac{(A_{560})\ (100)}{(\text{grams of sample})\ (1{,}000)(5)}$$

[39 FR 19076, May 30, 1974, as amended at 43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32607, Aug. 26, 1988; 53 FR 39839, Oct. 12, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.65 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline is [4S-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimeth-